Subcritical Water Extraction and Characterization of Polysaccharides and Phenolic Compounds from Inonotus obliquus

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（亜臨界水抽出法を用いたカバノアナタケから多糖類及びフェノール類化合物の抽出及びそれらの成分の特徴）

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論　文　の　要　旨
Abstract of thesis

This research explored the possibility and feasibility of subcritical water extraction (SWE) as a high-efficiency and environment-friendly extraction for polysaccharides (IOP), total phenolic content (TPC), and total flavonoid content (TFC) from *Inonotus obliquus* (*I. obliquus*). SWE processes have been optimized using response surface methodology (RSM) with a design by Box–Behnken design (BBD) under varying process conditions including extraction temperature, residence time and liquid-solid ratio. Besides, physicochemical characteristics such as FT-IR spectra, monosaccharide compositions, antioxidant activity and anticancer activities of extracts were determined and compared between the extracts by using SWE and hot water extraction (HWE). Furthermore, six kinds of phenolic ingredients (gallic acid, epigallocatechin, catechins, chlorogenic acid, vanillic acid, epicatechin and ferulic acid) were determined by HPLC. Overall, the results presented in this study not only establish accurate mathematical models of SWE process for obtaining functional ingredients from *Inonotus obliquus*, but also provide the theoretical foundation for the extensive application in the field of medical plants achieving huge economic, environmental and social benefits.

In chapter 1, the author introduced basic knowledge on the raw material, SWE, target compounds as well as the previous studies. This chapter introduced that SWE is an outstanding extracting method to selectively obtain a variety of polar or non-polar organic compounds (functional ingredients) as it can overcome several shortcomings over the conventional extraction methods (e.g. consuming time and biohazard).

In chapter 2, the author optimized the SWE procedures for polysaccharides extraction from *I. obliquus*. Depending on single factor experiments and RSM designed by BBD, the optimum IOP extraction conditions were confirmed. Considering the operation conditions for the SWE device, the parameters were modified slightly in the verification experiment as follows: extraction temperature of 194°C, residence time of 5.36 min, and liquid-solid ratio of 53 mL/g. The experimental extraction yield was 168.80 ± 0.59 mg/g, about 5.5 times

of that extracted by HWE (30.71 ± 0.43 mg/g).

In chapter 3, the author compared the physicochemical properties of IOP extracted by SWE and HWE. FTIR spectra analysis indicated that both samples contained neutral polysaccharides, exhibiting almost the same function groups and various stretching vibrations (O-H, C-H, C=O, -OH and C-O). Moreover, monosaccharide composition determination revealed that both polysaccharides were acidic polysaccharides with the major ingredient being glucose. The main monosaccharide compositions of HWE-IOP were glucose (60.18%) and galactose (18.02%), whereas those of SWE-IOP were glucose (82.49%), xylose (8.60%) and mannose (6.71%). Eight main monosaccharides including rhamnose, galactose, arabinose, glucose, xylose, mannose, galacturonic acid and glucuronic acid in HWE-IOP and SWE-IOP were 1.33: 4.75:1.13: 16.37: 1.85: 3.38: 0.48: 1.51 and 0.02: 0.27: 0.24: 35.53: 5.29: 4.61: 0.15: 0.78, respectively. The average Mw value of HWE-IOP was 66.51 kDa whereas the average Mw value of SWE-IOP was < 10kDa. Meanwhile, SWE-IOP exhibited stronger antioxidant activity than HWE-IOP, with IC50 being 0.86, 0.039 and 0.13 mg/mL respectively in DPPH radical scavenging activity, SOD-like activity and Hydroxyl radical scavenging activity assays in comparison to 1.78, 0.077 and IC50 0.41 mg/mL for HWE-IOP, most probably due to the changes of monosaccharide compositions and their molecular weights. The results also indicated that IOPs extracted by SWE and HWE at the concentration of 5 mg/mL, had little toxicity on human normal fibroblast (TIG-3) cells with cell viability of 84.73 ± 5.41% and 89.73 ± 3.97% for SWE-IOP and HWE-IOP, respectively. Meanwhile SWE-IOP exhibited a slightly stronger anti-proliferation effect on human alveolar basal epithelial (A549) cells with inhibition ratio of 89.76 ± 3.97% in comparison to 85.83 ± 5.08% for HWE-IOP.

In chapter 4, the author performed the multi-response optimization in terms of TPC and TFC of phenolic antioxidants from *I. obliquus.* The verification test revealed that the regression models were satisfactorily accurate for phenolic antioxidants production from *I. obliquus*. Experimental results showed that the experimental TPC of 477.58 ± 0.59 mg GAE/g dry chaga, TFC of 971.13 ± 0.42 mg RE/g dry chaga and ABTS radical scavenging activity of 178.34 ± 1.23 mM AAE/g were achieved at 209°C for 4.60 min with a liquid-solid ratio of 110.50 mL/g. In comparison, TPC of 121 ± 1.06 mg GAE/g dry chaga, TFC of 240 ±1.43 RE/g dry chaga and ABTS radical scavenging activity of 62.83 ± 0.76 mM AAE/g were determined at 40°C for 4 h with an ethanol concentration of 75%. In this chapter, six kinds of phenolic ingredients (expressed as mg/g dried plant) were determined by HPLC as follows: gallic acid of 0.24 mg/g, epigallocatechin of 8.57 mg/g, catechins of 17.84 mg/g, chlorogenic acid of 1.99 mg/g, vanillic acid of 1.07 mg/g, epicatechin of 13.33 mg/g and ferulic acid of 1.60 mg/g.

審　査　の　要　旨
Abstract of assessment result

This research investigated the possibility and feasibility of applying subcritical water extraction (SWE) in extraction of polysaccharides and phenolic compounds from *I. obliquus*. Compared with hot water extraction (HWE), the yield of polysaccharides by using SWE was enhanced by 5.5 times. Physicochemical characteristics such as FT-IR spectra, monosaccharide compositions, antioxidant activity as well as anticancer activity were determined and compared on the extracts from *I. obliquus* by using SWE and HWE. The bioactivity of extracts by SWE were much stronger than those obtained by HWE. Consequently, this study further analyzed the principle and advantages of SWE and its applications to achieve functional components (polysaccharides, total phenolic content, and total flavonoid content) from *I. obliquus*. Results from this research indicated that SWE could be a promising alternative for the extraction of antioxidant materials from *I. obliquus* since it not only significantly increases the extraction yield but also enhances the bioactivity of targets compounds.

The final examination committee conducted a meeting as a final examination on 1st August, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

Therefore, the final examination committee approved that the applicant is qualified to be awarded the degree of Doctor of Philosophy in Environmental Studies.